Hierarchical coexistence of universality and diversity controls robustness and multi-functionality in intermediate filament protein networks

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Proteins constitute the elementary building blocks of a vast variety of biological materials such as cellular protein networks, spider silk or bone, where they create extremely robust, multi-functional materials by self-organization of structures over many length- and time scales, from nano to macro. Some of the structural features are commonly found in a many different tissues, that is, they are highly conserved. Examples of such universal building blocks include alpha-helices, beta-sheets or tropocollagen molecules. In contrast, other features are highly specific to tissue types, such as particular filament assemblies, beta-sheet nanocrystals in spider silk or tendon fascicles. These examples illustrate that the coexistence of universality and diversity – in the following referred to as the universality-diversity paradigm (UDP) – is an overarching feature in protein materials. This paradigm is a paradox: How can a structure be universal and diverse at the same time? In protein materials, the coexistence of universality and diversity is enabled by utilizing hierarchies, which serve as an additional dimension beyond the 3D or 4D physical space. This may be crucial to understand how their structure and properties are linked, and how these materials are capable of combining seemingly disparate properties such as strength and robustness. Here we illustrate how the UDP enables to unify universal building blocks and highly diversified patterns through formation of hierarchical structures that lead to multi-functional, robust yet highly adapted structures. We illustrate these concepts in an analysis of three types of intermediate filament proteins, including vimentin, lamin and keratin.

Keywords: Hierarchical material, protein, universality, diversity, robustness, adaptation

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1. Introduction

Proteins constitute the elementary building blocks of a vast variety of biological materials such as cells, spider silk or bone, where they create multi-functional and highly robust structures, which - without wasting resources - arrive at satisfactory solutions [1-4]. Virtually all biological protein materials feature a decentralized organization [5-7], wherein self-organization, self-regulation, and self-adaptation govern the formation, reformation and repair or healing at multiple time- and length-scales.

Even though protein materials lead to vastly complex structures such as cells, organs or organisms, an analysis of their composition reveals simple underlying mechanisms that can be classified into two major categories. Some of the structural features materials are commonly found in different tissues, that is, they are highly conserved. Examples of such universal building blocks include alpha-helices, beta-sheets or tropocollagen molecules. In contrast, other features are highly specific to tissue types, such as particular filament assemblies, beta-sheet nanocrystals in spider silk or tendon fascicles [1]. These examples illustrate that the coexistence of universality and diversity is an overarching feature in protein structures. We believe that this is an important concept that characterizes the structure of protein materials, in the following referred to as the universality-diversity paradigm (UDP).

This paradigm is a paradox: How can a structure be universal *and* diverse at the same time? In protein materials, the coexistence of universality and diversity is enabled by utilizing hierarchies, which serve as an additional dimension, enlarging the 3D or 4D physical space.

Here we illustrate that the UDP is crucial to understand how structure and properties of protein materials are linked [8-10]. Through the UDP it is possible to improve our understanding of how protein materials are capable of robustly unifying seemingly incompatible features at different hierarchical scales.

We proceed as follows. In Section 2, we briefly review the definition of the most important terms used in this paper (such as hierarchies, robustness or simplicity), combining the terminology typically used in system theory and biology with concepts of materials science. In Section 3, we exemplify the UDP in a particular class of proteins called intermediate filaments (IFs). IFs form protein networks in the cytoskeleton of eukaryotic cell, stabilize the nuclear envelope and provide the basis for extra-cellular tissues such as hair or nails. Based on this example, we show how universality and diversity are combined through hierarchical material design, leading to highly adapted, robust and multifunctional structures, governed through self-regulatory processes. In Section 4, we generalize this concept and describe a generic framework that is applicable to a wide range of biological protein structures, and discuss these insights in light of a paradigm shift in material synthesis. In Section 5, we discuss the impact and challenges of the UDP. We illustrate the potential impact of a better understanding of hierarchical biological protein materials in the areas of materials science, engineering and other disciplines.

2. System theoretical perspective on biological structures

2.1 Hierarchies

Hierarchical systems have been observed already previously in many areas, including non-biological and biological areas. In system theory (ST), a hierarchical system is defined as a composition of stable, observable sub-elements that are unified by a super ordinate relation [11]. Thereby, lower level details in a complex hierarchical system may influence higher hierarchical levels and consequently affect the behavior of the entire system. Therefore, the interactions between different hierarchical levels or, equivalently, hierarchical scales are the focal point in ST based concepts of hierarchical systems.

Importantly, averaging over one scale to derive information for the next higher scale is generally not feasible. This is because either an insufficient number of sub-elements is present [12], or because a particular piece of information may be forfeited that might be crucial for the behavior several scales up [11]. This aspect is different from many engineering approaches that are based on the idea of coarse-graining or averaging over a specific microstructural unit cell and calculation of effective material parameters (see, for instance computational methods such as the quasicontinuum method [13-15]).

One of the best understood hierarchical systems is the "hierarchy of life" (HOL), where cells, organs, organisms, species, communities, and other entities are put together in an inclusive hierarchical relation [11]. However, in the HOL a cell is the smallest hierarchical subunit. In the last decades, several additional subunits ranging from cellular to the atomistic level have been discovered, including protein-networks and individual proteins, reaching down to the scale of the chemistry of individual amino acids.

The discoveries made on small scales (that is, at the protein levels) gave among others rise to a new discipline: the science of systems biology, where the focus lies on understanding a system's structure and dynamics, such as signaling cascades, as well as its emergence and control [7].

To facilitate the discussion in this paper, we have adapted some of the terminology from ST and put it into the system biological and materials science context. The most relevant terms for hierarchical biological materials (HBM) are explained in more detail in the following sections and summarized in Table 1.

2.2 Robustness and complexity

Biological materials and systems are critical elements of life. That's why it would be very harmful if the failure of a single component would lead to a catastrophic failure of the whole system. Thus a major evolutionary driving force in biological materials is to increase the robustness against the failure of a single component or a change of environmental conditions, in other words, the maintenance of some desired systems characteristics despite any fluctuations imposed by the environment.

Recently, Kitano classified the robustness of biological systems in three ways [7, 16]: (i) Adaptation, which denotes the ability to cope with environmental changes, representing the 'external perspective', (ii) parameter insensitivity, representing the internal perspective of robustness, and (iii) graceful degradation, reflecting the characteristic slow degradation of a system's function after damage, rather than catastrophic failure. These different dimensions of robustness build the equivalent to the fundamental, system theoretical aspects of robustness, which are phase tolerance and amplitude tolerance.

However, robustness has its costs. One means in realizing robustness is the use of redundancies, that is, many autonomous units carry out identical function. Examples are multiple genes that encode similar proteins, or multiple networks with complementary functions in cells [16]. While redundancies increase the robustness of a system, they also increase the system complexity. Therefore, higher degrees of complexity are partly believed to be additional costs of robustness. This agrees with the notion that biological systems are results of a trade-off between robustness and internal simplicity [4, 17-21].

Nevertheless, there is yet no consensus in the community whether or not biological systems are actually "complex". As illustrated above, parts of the community believe that complexity is necessary for robustness and thus essential for biological systems [17]. Others believe that "coherence" or "symbiosis" are attributes that describe biological systems in a better way than complex [16]. A third part of the community finds that biological systems are much simpler than we often assume, given the fact that cells evolved to survive, and not for scientists to understand [4, 5].

2.3 Simplicity, modularity and protocols

How does Nature solve the conflict between robustness and simplicity, while achieving a controlled degree of complexity? Applications of a limited number of universal building blocks, network motif or modules seem to be the path to success [4, 5]. Alon illustrates this simplicity on gene-regulation networks, which are build out of only a handful networks motifs [5]. But modularity does not only occur on the gene level. It plays an equally important role from base pairs and amino acids to proteins, from organelles and membranes to pathways and networks, and finally to organs and organ axes. Additionally, even complex processes, such as protein folding, have been shown to be much less complex than expected for along time [4, 6].

An additional source of simplification in biology is the strong separation of timescales for different processes. For instance, the production of proteins takes place on the time scale of minutes, while the chemical modification of protein networks is realized within short time scales that span only several seconds [5]. Individual bonds (e.g. H-bonds) form at time scales of tens of picoseconds.

Finally, Wolfram has indicated in his studies with simple programs that the degree of complexity in biological systems can be achieved through simple rules and elements [22]. Another word for rules is protocols, which are designed to managed relationships and processes, building the architecture, interfaces and etiquettes of systems. Thus, abstractions such as gene regulation, covalent modification, membrane potentials, metabolic and signal transduction pathways, action potentials, and even transcription-translation, the cell cycle, and DNA replication could all be reasonably well described as protocols [4]. Notably, the simplest protocols, those that control the behavior at the atomic scale, are the force fields describing the covalent and non-covalent interactions, such as hydrogen bonds, Coulomb interactions or van der Waals interactions.

In general, specific protocols describe the interaction between elements as well as between different scales in a hierarchical system. A good protocol is one that supplies both robustness and evolvability. Therefore, successful protocols become highly conserved because they both facilitate evolution and are difficult to change [4, 19]. This may be an important aspect in understanding the observation of universal features in protein materials – these may be related or represent protocols that are particularly successful.

2.4 Perfect adaptation and optimality, evolvability and recreation

The standard Neo-Darwinian theory of evolution is based on the idea that random genetic changes, coupled with natural selection, will result in progressive transformation of form, which can give rise to new structures and functions in organisms [23]. Protocols support this process of adaptation by activating ‘algorithms’ that facilitate the optimization of fitness functions. The result of this optimization process is a perfect adaptation towards different structural requirements [4, 17-20].

Perfect adaptation means maximal efficiency, which leads one to conclude that each element has its own place in a biological system. Once this element is taken out while its function is still activated, a new element is created, which will fulfill this particular function instead. A similar mechanism is activated when new functions appear. In other words, upcoming challenges are addressed by the generation of new elements or by the adaptation of existing ones. This mechanism of adaptation and (re-)creation has been proven for macroscopic biological systems (e.g. fruit fly species on Hawaii) [24].

Related observations have been made at much smaller, microscopic tissue scales, for instance in actin stress fiber generation or in the case of continuously adapting collagen networks. In many of these tissues, microscopic fibers are formed where needed and degraded elsewhere. These observations hint on the fact that maybe perfect adaptation and efficiency may be governing all micro- and nanoscopic structures and processes.

As demonstrated, system theory and system biology provide first significant insight into the properties of biological system. However, up until now, to the best of our knowledge there is no theoretical paradigm that describes such concepts from the viewpoint of materials science, for the case of hierarchical biological materials (HBM). This may have prevented researchers from fully appreciating and understanding the structure-property relationship of HBM, and has limited applicability of concepts found in HBM in technological applications, for instance in the creation of new synthetic nanomaterials. Most importantly, we hypothesize that structure and process must be integrated in comprehensive theories of HBMs.

3. Robustness and multi-functionality in intermediate filament proteins

In this Section, we illustrate for the particular case of IFs how universality and diversity, silencing and activation are combined in a hierarchical structure, building materials with multiple, scale specific functions, which on their part are combined with scale specific processes. An overview for the IF protein network is shown in Figure 1.

The lowest level of hierarchy encodes the structure of these proteins in the sequence of amino acids (AA). This is reflected by the fact that each IF type has a distinct AA sequence. Intriguingly, the differences at the lowest hierarchy do not influence the immediately following hierarchical level. This can be verified since all IFs feature the alpha-helical motif, despite differences at the AA sequence level and/or differences at larger scales. However, moderate effects can be observed at the dimer level. Herein, for example amino acid inserts in the periodic heptad repeat lead to a local uncoiling of the super helix (creating the stutter), which effects the assembly process as well as the unfolding mechanics [25, 26]. Another example is the occurrence of mutations in desmin IF coiled-coils. It was shown that disease related mutations do not destroy the AH structure but build additional stutters or stammers in the coiled-coil [27].

Even though all types of IFs commonly show an assembly into filaments, lower scale differences (that is, for instance the AA sequence and stutter) affect the pattern and process of assembly, such as the number of proteins per filament cross-sectional area, or the way dimmers associate. The differences on the filament level are of utmost importance, as they influence the properties at the network and the super-structural level, which are dominated but not limited to mechanical functions.

In the following examples, links between the hierarchical design and the resulting multiple functions and processes are discussed. The multiple functions of the different IF types are summarized in Table 2.

3.1 Vimentin networks in the cytoskeleton

Vimentin networks in the cytoskeleton act mainly as the 'security belts' of the cell [28, 29]. Due to their architecture, the flexible networks are very soft at small deformations and pulling rates, leading to 'invisibility' and non-resistance during active cell movement (governed by the dynamics of actin filaments and microtubules). Contrarily, a very stiff behavior is observed at high deformations and high deformation rates, ensuring their function on the cellular as well as on the tissue level [30].

Recently, additional functions have been found on the sub-network level (filament level), which are still but less mechanical. Vimentin networks were proved to be not only responsible for the location, shape and stability of cell organelles (e.g. mitochondria or golgi), but also for their function as well as for the protein targeting process [31]. And yet other function exist on the molecular level, consisting of different regulation mechanisms such as cell signalling (e.g. transcriptional effects, mechano transduction), or associated protein organisation (e.g. plectin, chaperones) [8, 31].

3.2 α-keratin networks in skin tissue, hair, nails and hoofs

Representing one of the main cytoskeletal components in skin epithelia cells [32, 33], keratins fulfill similar structural functions as vimentin, which are, protecting cells from mechanical and non-mechanical stresses, enabling cell signaling, or organizing cell organelles and keratin associated proteins. But that is by far not all.

In addition, evidence was reported that keratins are responsible for several skin cell specific processes such as cell pigmentation (hyper- or hypo-pigmentation of the skin due to keratin mutations), cell growth, protein synthesis and wound healing (controlled through keratin signaling chains) [33, 34], providing strong evidence of adaptation of this protein structure towards additional functional requirements on the surface of organisms.

Even more fascinating is that α-keratins also build the main component of hair, nails, hoofs and claws (and β-keratins are the main component of the even harder materials such as turtle shells or bird beaks), where micro- and macro fibrils are embedded in a sulfur rich matrix [35-38]. This enables the material to provide significant macroscopic mechanical resistance for locomotion or prey procurement.

3.3 Lamin networks in the nuclear envelope

The case of lamins is slightly different than the two previous examples, because lamins are associated with the inner nuclear membrane of cells, where they provide a dense and resistant network against compression [39, 40]. This architecture enables them to realize their mechanical function, which is to protect chromatin in the nucleus from mechanical load. Diseases related to mutations in lamins, such as skeletal or cardiac myopathies (e.g. Emery-Dreifuss muscular dystrophy), which among other effects leads to uncontrolled rupture of the nuclear envelope, resulting in cell death [41].

However, similar to the previous cases the role of lamins is not purely structural. In addition to the structural hypothesis, the 'gene regulation hypothesis' is gaining a broader acceptance, which gives lamins a key role in the organization of DNA as well as in the gene transcription process [41-44]. Further, lamins are suggested as one key element in the signaling chain, forwarding signals from the cell-membrane to the DNA, where a specific response is triggered [44]. This exemplifies how structure and property are linked.

3.4 Coexistence of universality and diversity

The case of IFs illustrates how hierarchies are applied in order to unify universal robust elements (AHs) and highly diversified and optimized patterns (specific head-tail domains, network architecture, and others). As shown in this example (see Figure 1), nanoscopic modifications do not always influence the properties at the next hierarchical layer, but those of one ore more hierarchical layers above.

It appears as if specific functional requirements at several higher scales are ‘forwarded’ to lower scales, where modifications are implemented. Through this mechanism biological materials are not only multi-functional but are further continuously adapted to the required scale-specific processes, with the goal to fit the diverse required functions in the best possible way.

4. Generic paradigm: Linking universality and diversity through hierarchical structure-design

As illustrated on the example of IFs, but in principle also applicable to other protein materials such as beta-sheets crystals in spider silk or bone, HBM are a great source of scientific and technological inspiration. They show that hierarchical design is an essential feature in Nature, enabling to unify synergistically contradictive dimensions (e.g. universe/diverse, global/local), resulting in multi-functional biological materials with adapted (e.g. on the assembly level), yet robust (e.g. individual alpha-helices) properties.

However, up to now no theoretical framework is present that enables to address relevant questions in HBM systematically within a unified multi-perspective approach. With the generic universality-diversity paradigm summarized in Figure 2 we hope to close this gap.

4.1 Unifying strength with robustness through hierarchies

Csete and Doyle have claimed that optimality and robustness are most important for the properties and behavior of biological systems [4]. But how does this relate to HBMs? We believe that from the mechanical point of view, the parameter ‘strength’ has to be optimized and thus corresponds to optimality in this context.

The properties 'strength' and 'robustness' (see previous section for definition) are contradicting properties that can not be combined within a single scale of 'traditional' materials. This can be demonstrated by considering a simple cubic crystal lattice. The strength of the lattice is characterized by the atomic interactions. In order to display a large strength of the material, the atomic bonds need to be strong and break at large interatomic forces. However, this leads to a very brittle and thus fragile material, like glass, that can not be deformed under large load. Contrarily, to make materials more robust in order to prevent it from catastrophic failure the atomic bonds must have the properties which enable an easy reconfiguration, leading to effortless shearing of the lattice. But then, the material becomes ductile like a piece of a very soft metal that can easily undergo shape changes [45-47].

Many materials and structures engineered by humans bear such a conflict between strength and robustness; strong materials are often fragile, while robust materials are soft. Fragility appears due to the high sensitivity to material instabilities such as formation of fractures [48, 49]. Consequently, only high safety factors and thus bigger amount of resources can guarantee the strength of engineered materials, if extreme conditions are expected [47, 50].

This example illustrates that it is difficult or impossible to combine strength and robustness at a single scale; instead, structures with multiple scales must be introduced, where universal and divers patterns are unified hierarchically. In these structures, universality generates robustness, while diversity enables optimality. Materials like bone, being a nano-composite of strong but brittle and soft but ductile materials, illustrate this unification of components with disparate properties within a hierarchical structure [51-54].

Obviously, extreme mechanical conditions (such as high loading rates and large deformation) have to be sustained in Nature under limited access to 'building materials', which make the combination of strength and robustness imperative for existence. Therefore, materials found in biology are very efficient due to robustness, and thus capable of minimizing waste of resources that otherwise appears from high safety factors.

Notably, optimality might also appear in a non-mechanical sense, such as optimized thermal, electrical or energy organization and conductivity.

4.2 Controlling properties through silencing and activation

Particular features of HBMs are silencing and activation mechanisms acting on different scales. These mechanisms represent a set of ‘tools’ that provide the ability for local optimization while simultaneously guaranteeing global robustness.

Robustness is guaranteed when differences or changes that appear at the lower hierarchical scale do not influence higher scales (e.g. alpha helices), that is, expressing *silencing* (robustness in the sense of parameter insensitivity), which allows a global application of this particularly stable feature.

In contrast to that, if an element has great potential to *activate* larger scale properties, that is, its changes appear ‘nonlocal in scale’, its application is not ‘safe’ and conservation is unlikely. Given that systems that are robust against common or known perturbations can often be fragile to new perturbations [16-18], it is not surprising that these ‘unsafe’ features are extensively applied whenever self-optimization and continuous adaptation are necessary (robustness in the sense of environmental adaptation). This aspect might explain why universal patterns are more often found on a lower hierarchical level, whereas diversified patterns appear at higher scales.

Remarkably, the question of local versus global changes seem to be relevant not only for HBM but also for other processes, such as gene regulation [55], illustrating that this is an overarching paradigm in biology.

4.3 Unifying multi-functionality with controlled complexity

Modern engineered structures and systems (e.g. air planes, cars or buildings) now reach a similar degree of multi-functionality as biological systems [4]. However, many engineered multi-functional structures have an uncontrollable degree of complexity, since a multitude of distinct elements are combined on a single or few hierarchical levels. Human organizations, in contrast, realize multi-functionality through hierarchical, but yet highly complex structures.

Approaches to create self-organized systems, such as the internet (or ‘the grid’), which are based on a standardized ‘protocol’ are simple yet fragile. This fragility is observed when bugs in the software appear, or viruses and spam (or certain types of overloads) spread very rapidly, without noticeable resistance. This is because these viruses utilize mechanisms that are compatible with the particular protocols in the network, and decrease the efficiency of or even knock out entire networks [56, 57].

In contrast to these examples, Nature follows a different path. Here, multi-functionality is created through hierarchically combining universal and robust patterns on particular levels with diversified, but optimized or adapted elements on others. This results in robust and multi-functional, yet simple systems, where complexity is kept under control, making the structure as whole more efficient. Instead of reinventing new building blocks, universal patterns and protocols (e.g. the kind of interatomic bonding) are utilized and ‘internal degrees of freedom’ arising from lower scales are kept or conserved. These degrees of freedom are ‘forwarded’ to higher scales, where application is necessary. This concept of silencing enables to adapt systems without significantly changing them, and appears to be a universal trait of biological systems.

4.4 Decentralized processes

Remarkably, in contrast to Nature’s structural design, which is dominated by hierarchies, Nature’s *process* design is dominated through decentralization and self-organization, represented through self-assembly, self-regulation, self-adaptation, self-healing and other processes (see Figure 2).

Interestingly, the decentralized processes seem to lead to a multi-scale perspective in time, where different time scales are covered, ranging from several picoseconds for creation of individual H-bonds, over minutes for assemblies and rearrangement, to eons for adaptation and optimization. The separation of processes through different time scales makes also sense from the biological point of view, as these increase both simplicity and robustness (see also the introduction section).

4.5 Linking structure and process

As indicated in Figure 3, we believe that in biological materials hierarchical structures, decentralized processes, material properties and environmental requirements, are brought together in a mutual completion.

In contrast to the traditional paradigm in materials science, relations between “external” functions/requirements and “internal” properties exist on several scales resulting in multi-functionality. Though, as requirements are consistently changing (e.g. changing loads, changing environment) on several time and length scales, in addition to multi-functionality, robust feedback loops are required and enable decentralized self-organization and self-optimization.

This clearly shows that in HBM structures and processes are amalgamated and can not be considered alone.

5. Discussion

The UDP is of vital importance from a scientific, technological as well as sociological perspective. The UDP offers a pathway to understanding some of the challenging properties of HBM in a systematic way, enabling its transformation into technological development. The UDP could be used to formulate research questions and address such issues in a directed fashion.

We suggest the following possible path to success. First of all, the UDP provides a theoretical framework, which enables to define future scientific hypotheses in the field of HBM in a systematic way. These hypothesis must be proved in a second step through a unified approach that combines theory, experiment and simulation, leading to improved understanding in two main dimensions: (i) a detailed understanding of hierarchical design laws, such as cross scale interaction and cross-scale integration, and (ii) a detailed understanding of how Nature successfully links structure, processes, properties and functions simultaneously over many length scales, from nano to macro.

The collaboration between materials scientists and structural biologists is vital and will be mutually beneficial: Materials scientists have extensive of experience in treating structures, processes and properties of materials systematically and with rigorous mathematical methods. On the other hand, biologists have gained a detailed understanding of biological systems and structures and related functions. This will lead to a better understanding and new theories, which will build the foundation for the design of synthetic hierarchical structures and systems.

5.1 Impact on other scientific disciplines

Detailed analysis of HBM with the help of UDP may contribute to a variety of scientific disciplines, such as the science of fracture, materials theory, genetic research (e.g. the hierarchical three dimensional folding of the DNA). In these examples, the link between structural organization and function [58] is a vital component that might further contribute to the understanding of which driving forces in Nature create hierarchical biological materials.

Additionally, the UDP might integrate different scientific strategies (e.g. macroscopic [25-27] versus nanoscopic [59-62] approaches in understanding fracture of bone), through the holistic consideration of problems, using the concept of coexistence of universality and diversity at different scales and application of both through fundamental design laws.

The theoretical progress in understanding HBMs will enable us to use the extended physical space in an efficient and controlled manner, that is, leading to a bottom-up structural design on the sub-macroscopic scale, instead of blind trial-and-error approaches. For example, the extended design space might serve as a means to realize new physical realities that are not accessible to a single scale, such as material synthesis at moderate temperatures, or fault tolerant hierarchical assembly pathways [24], which enable biological systems to overcome the limitations to particular chemical bonds (soft) and chemical elements (organic) present under natural conditions.

The increased understanding of the hierarchical design laws might further enable the development and application of new organic and organic-inorganic multi-featured composites (such as assemblies of carbon nanotubes and proteins or polymer-protein composites [63-65]), which will mainly consist of elements that appear in our environment in a practically unlimited amount (C, H, N, O, S). A better use of these materials might consequently help us to address important energy and resource problems (e.g. fossil resources, iron and others), and allow us to manufacture nano-materials, which will be produced in the future by techniques like recombinant DNA [66-68] or peptide self-assembly [69-71] techniques, where the boarders between materials, structures and machines vanish.

Elucidation of the controlling factors in achieving universality and diversity, as well as the understanding of its impact on robustness and adaptation and optimality, could lead to a paradigm shift that emphasizes on simultaneous control of structural features at all length scales and hierarchies.

Engineers will be able to design smart sensor-actuator networks on nano-scale, which will enable chemo-mechanical transduction, leading to self-organization and adaptation to the environment. These networks will be part of micro-machines, which will be able to perform complicated tasks in a robust and secure way. These machines, being part of higher order structures, will enable self-adaptation, self-strengthening and self-repair through their high level of cooperation.

Further, a detailed understanding of HBM and the generation of appropriate HBM models from cells and extracellular tissues with a particular focus on the link between structures, functions and processes, as well as cross-scale interaction and interscale connection, could lead to immense progress in the rising field of nano-medicine and thus influence other industries such as the pharmaceutical and cosmetic industry. Specific examples of applications include improved drug delivery systems or the development of methods that facilitate *in vivo* tissue repair processes.

In more general terms, researching hierarchical protein materials through the eye of the UDP will provide a fundamental understanding of the question of repeated use of templates versus the making of new structures or components and its assembly in hierarchical structures. This might inspire future product design as well as manufacturing and assembly strategies. Using universal patterns to the fullest extent and creating diversity at the highest hierarchical level, in order to match client-specific requirements, will reduce production costs, delivery times at continuously high product quality.

It has been suggested that the complexity of engineered systems is converging with the one of biological systems. For example, a Boeing 777 has 150,000 subsystems and over 1000 computers, which are organized in networks of networks [4]. Consequently, a better understanding of how nature designs and manages complexity will enable to maintain or limit engineered complexity or even reduce it.

An extended understanding of the UDP paired with hierarchical multi-scale modeling and petaflop computing may have additional implications beyond scientific and engineering disciplines, such as creation and optimization of infrastructure networks (e.g. energetic, communication), organization or transportation systems, and others. Similar to engineered systems, new ideas and approaches will reduce the complexity of these structures by simultaneously increasing robustness and adaptability/flexibility – both crucial attributes in today's quickly changing world. Thus adaptive organizations and networks will lead to a better performance and consequently to a continuous economic growth, while the robust way these systems operate will increase the satisfaction and well-being of employees and citizens.

Significant impact could also be achieved in urban area design [72]. Hierarchically organized regions and cities, where the functional links between the sub-elements are inspired by biology, could for example address the traffic problem in large metropolitan areas, or dramatically slow down the spreading speed of epidemics [73]. These could be intriguing applications of nanoscience to large-scale problems.

5.2 Theoretical, computational and experimental challenges

In order to realize the promising opportunities that arise from an improved understanding of HPM, several critical challenges must be overcome.

Up until now, theories, describing hierarchical biological materials are still lacking. Virtually no understanding exists about how specific features at distinct scales interact, and for example, participate in mechanical deformation. However, such models are vital to arrive at a solution for the universality-diversity question and Nature's hierarchical material concepts. The path to success is to develop cross-scale relationships and constitutive equations for different hierarchical scales within the structure-property paradigm of materials science (see Figure 3), that is, to understand if and how nano-/meso-/micro-changes affect properties at larger scales. To achieve this goal, structural architecture will have to be considered across the scales, possibly combined with fractal theory [74], and investigated in light of the UDP.

Furthermore, the nomenclature for hierarchical biological materials is still missing. Definitions and measures for material properties such as hierarchical degree, level of robustness, degree of universality, and others, are crucial. Appropriate terminology for cross scale relations such as scale separation, -integration and -interaction must be defined. We hope that the UDP will stimulate extensive research in these directions.

Computational modeling techniques have progressed enormously during the last few years, and simulation techniques like MD find broad application and increasing acceptance. But these simulation approaches are still limited to samples of a few nanometers in size and modeling techniques, linking atomistic to continuum scale in biological materials, which lack a regular atomic lattice, are in a very early stage of development. To overcome these limitations, new numerical models will be necessary, followed by new approaches of data analysis and visualization methods.

In addition to the computational techniques, experimental techniques on the level of individual molecules progressed immensely during the last decade. Beyond experimental challenges, several manufacturing challenges need to be overcome, such as the application of recombinant DNA techniques to sustain industrial volumes, or the construction of macro-materials from nano-devices.

5.3 Concluding remarks

Overcoming these challenges will require a convergence of scientific disciplines in two regards. First, experimental, theoretical and computational approaches will need to be combined extensively, in order to understand, explain and successfully apply observed phenomena that are present in the biological nano-world. Along with the development of new technologies, it is vital to assess and minimize the risk associated with nano- and biotechnologies. Second, different disciplines like Physics, Chemistry, Biology, Engineering, Computer Science and Medicine will have to work together in an integrated manner. Each of these fields is indispensable for the understanding of the biological nanoscience and the future application of the generated knowledge in new technologies. Even a transition from a multi-disciplinary approach to the creation of a new discipline and scientific organizations is conceivable.

Historically, humans have first exploited natural materials, such as stone, wood and clay. Later, with the advent of Bronze and Iron Ages, metallurgy and synthetic materials have become more dominant. However, due to the limited resources, new approaches and inspirations are necessary [8]. Biological materials seem to be a conspicuous starting point for new directions that combines advances in nanoscience and nanotechnology towards the development of new materials.

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References

1. Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P., *Molecular Biology of the Cell*. Taylor & Francis: 2002.
2. Ball, P., *Made to Measure: New Materials for the 21st Century*. Princeton University Press: Princeton, N.J., USA, 1997.
3. Doyle, J. *Nature* **2007,** 446, 860.
4. Csete, M. E.; Doyle, J. C. *Science* **2002,** 295, (5560), 1664-1669.
5. Alon, U. *Nature* **2007,** 446, (7135), 497-497.
6. Baker, D. *Nature* **2000,** 405, (6782), 39-42.
7. Kitano, H. *Science* **2002,** 295, (5560), 1662-1664.
8. Bruck, H. A.; Evans, J. J.; Peterson, M. L. *Experimental Mechanics* **2002,** 42, (4), 361-371.
9. Ackbarow, T.; Chen, X.; Keten, S.; Buehler, M. J. *P. Natl. Acad. Sci. USA* **2007,** in press.
10. Ackbarow, T.; Buehler, M. J. *Journal of Materials Science* **2007** (available online), DOI 10.1007/s10853-007-1719-2.
11. Ahl, V., Allen, T.F.H, *Hierarchy Theory - A Vision, Vocabulary, and Epistemology*. Columbia University Press: New York, 1996.
12. Lakes, R. *Nature* **1993,** 361, (6412), 511-515.
13. Tadmor, E. B.; Ortiz, M.; Phillips, R. *Phil. Mag. A* **1996,** 73, 1529.
14. Knap, J.; Ortiz, M. *J. Mech. Phys. Sol.* **2001,** 49, (9), 1899-1923.
15. Dupuy, L. M.; Tadmor, E. B.; Miller, R. E.; Phillips, R. *Physical Review Letters* **2005,** 95, (6).
16. Kitano, H. *Nature* **2002,** 420, (6912), 206-210.
17. Carlson, J. M.; Doyle, J. *Proceedings of the National Academy of Sciences of the United States of America* **2002,** 99, 2538-2545.
18. Carlson, J. M.; Doyle, J. *Physical Review E* **1999,** 60, (2), 1412-1427.
19. Alon, U.; Surette, M. G.; Barkai, N.; Leibler, S. *Nature* **1999,** 397, (6715), 168-171.
20. von Dassow, G.; Meir, E.; Munro, E. M.; Odell, G. M. *Nature* **2000,** 406, (6792), 188-192.
21. Lezon, T. R.; Banavar, J. R.; Maritan, A. *Journal of Physics-Condensed Matter* **2006,** 18, (3), 847-888.
22. Wolfram, S., *A New Kind of Science*. Wolfram Media: Champaign, 2002.
23. Goel, N. S., Thompson, R.L., *Computer Simulations of Self-Organization in Biological Systems*. Croom Helm: London & Sydney, 1988.
24. Holland, J. H., *Hidden Order - How Adaptation Builds Complexity*. Helix Books: Reading, MA, 1995.

25. Ackbarow, T., Buehler M.J., . *under submission* **2007**.
26. Strelkov, S. V.; Schumacher, J.; Burkhard, P.; Aebi, U.; Herrmann, H. *Journal of Molecular Biology* **2004,** 343, (4), 1067-1080.
27. Kaminska, A. *Human Genetics* **2004,** 114, (3), 306-313.
28. Herrmann, H.; Aebi, U. *Annual Review of Biochemistry* **2004,** 73, 749-789.
29. Mucke, N.; Kirmse, R.; Wedig, T.; Leterrier, J. F.; Kreplak, L. *Journal of Structural Biology* **2005,** 150, (3), 268-276.
30. Mucke, N.; Kreplak, L.; Kirmse, R.; Wedig, T.; Herrmann, H.; Aebi, U.; Langowski, J. *Journal of Molecular Biology* **2004,** 335, (5), 1241-1250.
31. Toivola, D. M.; Tao, G. Z.; Habtezion, A.; Liao, J.; Omary, M. B. *Trends in Cell Biology* **2005,** 15, (11), 608-617.
32. Coulombe, P. A.; Omary, M. B. *Current Opinion in Cell Biology* **2002,** 14, (1), 110-122.
33. Gu, L. H.; Coulombe, P. A. *Current Opinion in Cell Biology* **2007,** 19, (1), 13-23.
34. Kim, S.; Wong, P.; Coulombe, P. A. *Nature* **2006,** 441, (7091), 362-365.
35. Zahn, H.; Fohles, J.; Nienhaus, M.; Schwan, A.; Spel, M. *Industrial & Engineering Chemistry Product Research and Development* **1980,** 19, (4), 496-501.
36. Kreplak, L.; Doucet, J.; Dumas, P.; Briki, F. *Biophysical Journal* **2004,** 87, (1), 640-647.
37. Kreplak, L.; Franbourg, A.; Briki, F.; Leroy, F.; Dalle, D.; Doucet, J. *Biophysical Journal* **2002,** 82, (4), 2265-2274.
38. Lorenzo Alibardi, M. T. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* **2007,** 308B, (2), 200-208.
39. Dahl, K. N.; Discher, D. E.; Wilson, K. L. *Molecular Biology of the Cell* **2004,** 15, 119A-120A.
40. Rowat, A. C.; Lammerding, J.; Ipsen, J. H. *Biophysical Journal* **2006,** 91, (12), 4649-4664.
41. Lammerding, J.; Schulze, P. C.; Takahashi, T.; Kozlov, S.; Sullivan, T.; Kamm, R. D.; Stewart, C. L.; Lee, R. T. *Journal of Clinical Investigation* **2004,** 113, (3), 370-378.
42. Lammerding, J.; Fong, L. G.; Ji, J. Y.; Reue, K.; Stewart, C. L.; Young, S. G.; Lee, R. T. *Journal of Biological Chemistry* **2006,** 281, (35), 25768-25780.
43. Mounkes, L.; Kozlov, S.; Burke, B.; Stewart, C. L. *Current Opinion in Genetics & Development* **2003,** 13, (3), 223-230.
44. Bridger, J. M.; Foeger, N.; Kill, I. R.; Herrmann, H. *Febs Journal* **2007,** 274, (6), 1354-1361.
45. Buehler, M. J. *Journal of Computational and Theoretical Nanoscience* **2006,** 3, (5), 603–623.
46. Buehler, M. J.; Ackbarow, T. *Materials Today* **2007,** 10, (9), 46-58.
47. Buehler, M. J.; Gao, H., *Ultra large scale atomistic simulations of dynamic fracture*. American Scientific Publishers (ASP): 2006.
48. Buehler, M. J.; Gao, H. *Nature* **2006,** 439, 307-310.
49. Buehler, M. J.; Abraham, F. F.; Gao, H. *Nature* **2003,** 426, 141-146.
50. Case, J., Chilver, L., Ross C.T.F., *Strength of Materials and Structures*. 4th ed.; Arnold New York, 1999.
51. Weiner, S.; Wagner, H. D. *Annual Review Of Materials Science* **1998,** 28, 271-298.
52. Currey, J. D., *Bones: Structure and Mechanics*. Princeton University Press: Princeton, NJ, 2002.
53. Buehler, M. J. *Nanotechnology* **2007,** 18, 295102.
54. Taylor, D.; J.G., H.; Lee, T. C. *Nature materials* **2007,** 6, (4), 263-266.
55. Berger, S. L. *Nature* **2000,** 408, (6811), 412-415.

56. Doyle, J. C.; Alderson, D. L.; Li, L.; Low, S.; Roughan, M.; Shalunov, S.; Tanaka, R.; Willinger, W. *Proceedings of the National Academy of Sciences of the United States of America* **2005,** 102, (41), 14497-14502.
57. Doyle, J.; Csete, M. *Nature* **2007,** 446, (7138), 860.
58. Fraser, P., Bickmore, W. *Nature* **2007,** 447, (7143), 413-417.
59. Gao, H. J. *International Journal Of Fracture* **2006,** 138, (1-4), 101-137.
60. Ji, B. H.; Gao, H. J. *Journal Of The Mechanics And Physics Of Solids* **2004,** 52, (9), 1963-1990.
61. Gao, H.; Ji, B.; Jäger, I. L.; Arzt, E.; Fratzl, P. *P. Natl. Acad. Sci. USA* **2003,** 100, (10), 5597-5600.
62. Gupta, H. S.; Seto, J.; Wagermaier, W.; Zaslansky, P.; Boesecke, P.; Fratzl, P. *P. Natl. Acad. Sci. USA* **2006,** 103, 17741-17746.
63. Cui, X. Q.; Li, C. M.; Zang, J. F.; Zhou, Q.; Gan, Y.; Bao, H. F.; Guo, J.; Lee, V. S.; Moochhala, S. M. *Journal of Physical Chemistry C* **2007,** 111, (5), 2025-2031.
64. Hule, R., A., Pochan, D., J.,. *MRS Bulletin* **2007,** 32, (4), 5.
65. Winey, K. I., Vaia R.A.,. *MRS Bulletin* **2007,** 32, (4), 5.
66. Langer, R.; Tirrell, D. A. *Nature* **2004,** 428, (6982), 487-492.
67. Petka, W. A.; Harden, J. L.; McGrath, K. P.; Wirtz, D.; Tirrell, D. A. *Science* **1998,** 281, (5375), 389-392.
68. Smeenk, J. M.; Otten, M. B. J.; Thies, J.; Tirrell, D. A.; Stunnenberg, H. G.; van Hest, J. C. M. *Angewandte Chemie-International Edition* **2005,** 44, (13), 1968-1971.
69. Zhao, X. J.; Zhang, S. G. *Macromolecular Bioscience* **2007,** 7, (1), 13-22.
70. Zhao, X. J.; Zhang, S. G. *Chemical Society Reviews* **2006,** 35, (11), 1105-1110.
71. Mershin, A.; Cook, B.; Kaiser, L.; Zhang, S. G. *Nature Biotechnology* **2005,** 23, (11), 1379-1380.
72. Webb, R. *Nature* **2007,** 446, (7138), 869.
73. Ferguson, N. *Nature* **2007,** 446, (7137), 733-733.
74. Mandelbrot, B. B. *Proceedings of the Royal Society of London Series a-Mathematical Physical and Engineering Sciences* **1989,** 423, (1864), 3-16.

Tables and table captions

Hierarchical systems	A hierarchical system is a system composed of stable, observable sub-elements that are unified by a super ordinate relation. Thereby, lower level details affect higher levels and thus the overall system behavior.
Complexity	Complexity arises in systems that consists of many interacting components and leads to emerging nonlinear behavior of a system. There is still no consensus if biological systems are complex or not.
Robustness	The following three classes of robustness are suggested to be relevant for biological system: (i) adaptation to environmental changes (external perspective), (ii) parameter insensitivity (internal perspective) and (iii) graceful degradation after system failure rather than catastrophic failure.
Protocols	Protocols are rules, which are designed to manage relationships and processes, building the architecture and etiquettes of systems. They are linking different elements as well as different hierarchies in a system.
Optimality and perfect adaptation	It is commonly believed that random changes in (biological) systems, supported by protocols give rise to new structures and features, leading to a continuously improved performance of a system, which finally results in perfect adaptation of the system and optimal fulfillment of a required function.

Table 1: Summary of a selection of system theoretical terms and concepts used in this paper.

IF type	Found in…	Functions		
		Protein level	Filament level	Cellular/ network level
Vimentin	Cell's cytoskeleton	cell signaling mechanisms, associated protein organization	responsible for location, shape and stability of cell organelles, protein targeting processes	security belt' of the cell
Keratin	Cytoskeleton, hair, nails, hoofs	protein synthesis, cell signaling mechanisms, associated protein organization	cell pigmentation, organization of cell organelles	cell growth, wound healing, locomotion, prey procurement
Lamin	Nuclear envelope	signaling mechanisms, mechano transduction, chromatin positioning	gene regulation and transcription, chromatin positioning	protection of the chromatin, involved in cell mitosis

Table 2: Intermediate filaments are remarkable due to their diverse appearance in organisms, where they fulfill multiple functions at different hierarchical levels. Interestingly, the elementary building block of all kind of IFs is identical - the universal alpha-helical coiled-coil motif.

Figures and figure captions

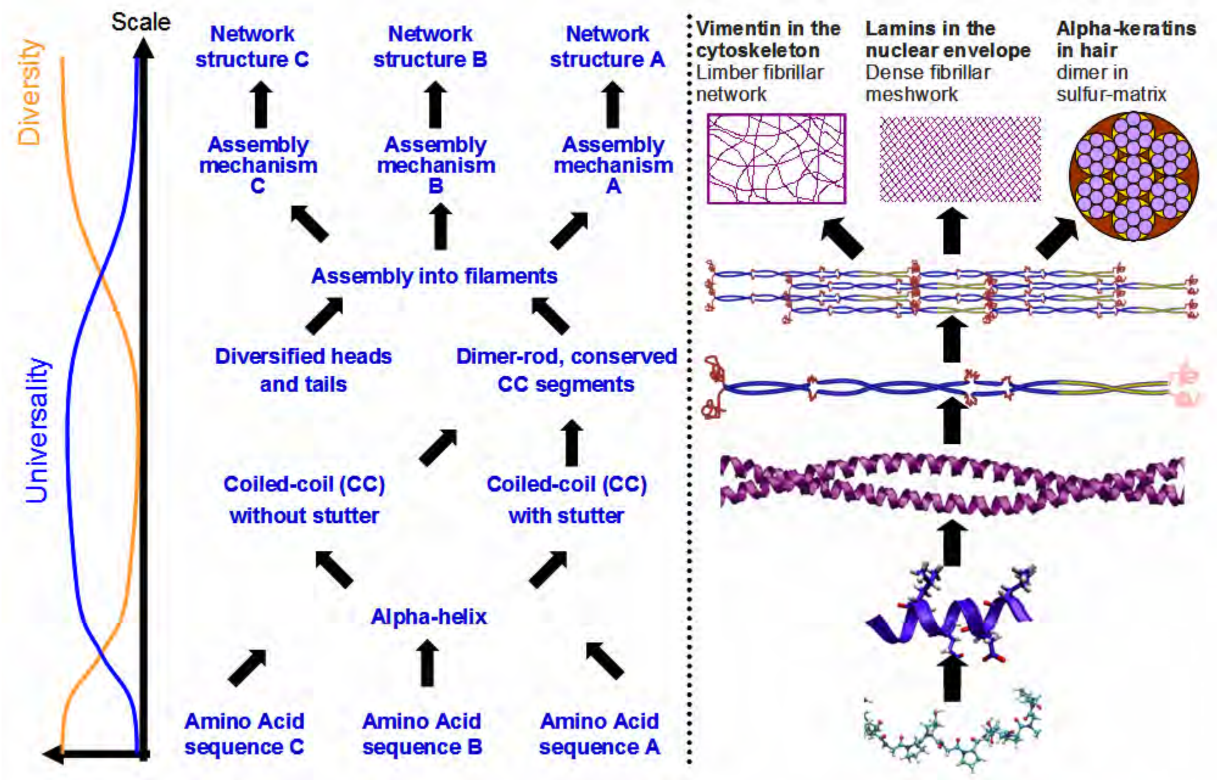

Figure 1: Hierarchical biological materials, here exemplified for the example of intermediate filaments (IFs), are governed through interplay of universal and diverse patterns, which, combined with silencing and activation are unified over multiple hierarchical scales. This enables to forward information that is completely coded at the lowest scale (amino acid sequence), safely by means of silencing through intermediate scales (alpha helix, coiled-coil) up to higher scales, where they are activated in order to fulfill specific requirements. The scale-characteristic patterns are illustrated on the right side.

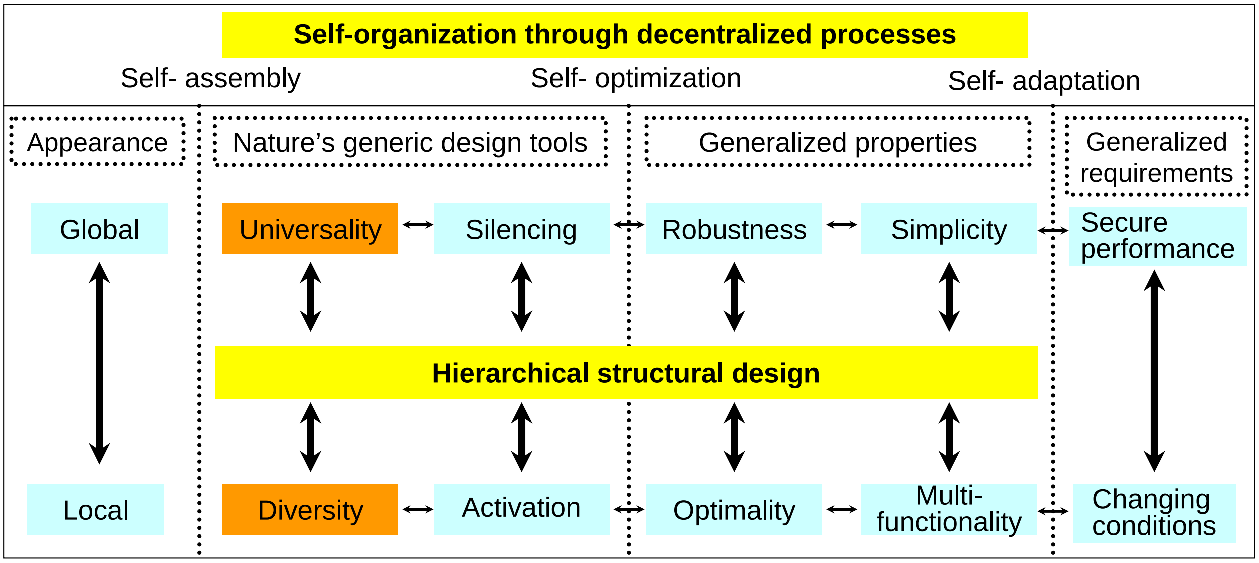

Figure 2: Hierarchical biological materials (HBMs) consist of two main perspectives: the hierarchical design on the one hand and the decentralized organization of processes on the other hand. The universality-diversity paradigm (UDP) allows addressing HBMs in a structured manner. Thereby, the processes are characterized through decentralized self-organization, including but not limited to: self-assembly, self optimization and self-adaptation, which can be realized through hierarchies, an additional dimension, effectively extending the 3D/4D physical space. Only this enlargement guarantees a synergized unification of seemingly un-linkable attributes of nature's tool box, which is necessary to realize 'generalized properties', which are required to fulfill specific functions as required from the environment, among other by the need to survive.

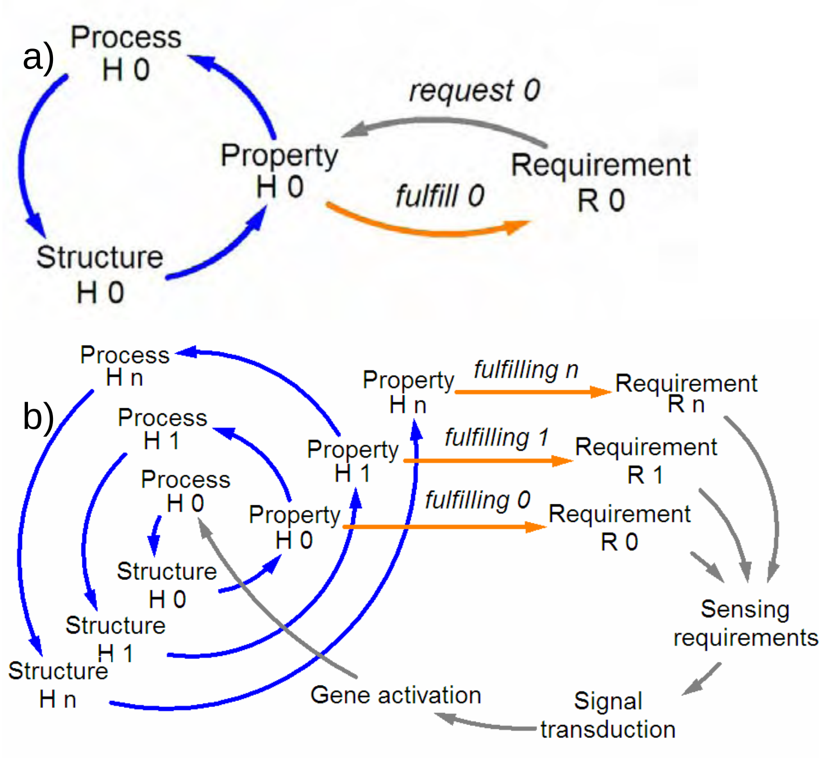

Figure 3: In biological materials hierarchical structures, decentralized processes, material properties and environmental requirements, are brought together in mutual completion. *Subplot a)* illustrates the traditional paradigm in materials science where process, structure and property build the "magic" triangle on a single hierarchical level. *Subplot b)* illustrates the paradigm for hierarchical (biological) materials. In contrast to the traditional paradigm, relations between "external" functions/requirements and "internal" properties exist on several scales resulting in multi-functionality. Further, as requirements are consistently changing over time (e.g. changing loads, changing environment), continuous adaptation is necessary. In addition to multi-functionality, robust feedback loops that result in smart signaling chains allow decentralized self-organization. Consequently, in HBM level-specific properties (H_i) do not only fulfill the required functions, but also initiate the decentralized processes on the next hierarchical level (H_{i+1}), and thus generate the structures on this level (H_{i+1}).

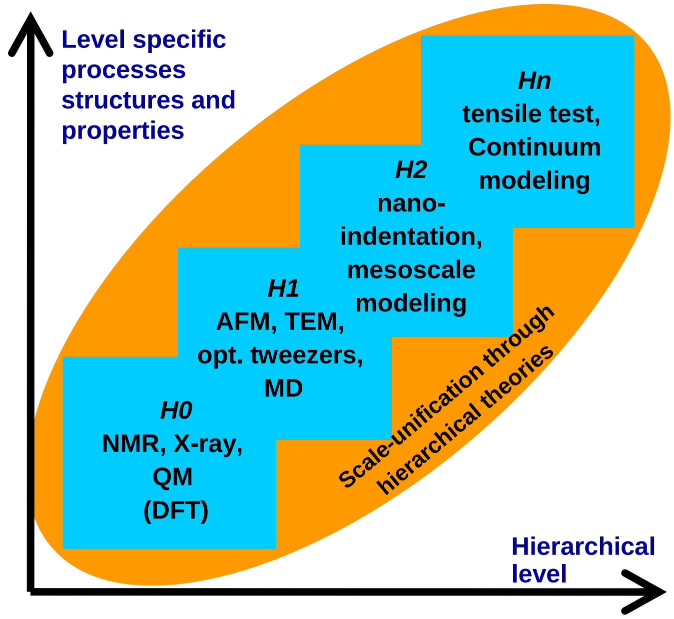

Figure 4: As shown in the previous figure, each hierarchical level in biological materials has its specific processes, structures and properties. In order to gain a detailed understanding of HBM on each scale as well as of the interaction between different hierarchies, theory, simulation and experiment will have to work together extensively. While simulation and experimental techniques are mostly limited to a certain length scale and so to a few hierarchical levels, new theories, fitted with information and knowledge from different hierarchical levels will describe the fundamental cross-scale relations and thus give explanations for observations on different scales.